SS A

- (ii) activating the resultant nuclear transfer unit;
- (iii) culturing said activated nuclear transfer units until greater than the 2-cell developmental stage; and
- (iv) culturing cells obtained from said cultured NT units to obtain embryonic stem-like cells.

AZ

- 15. (Amended) The method of Claim 1, wherein the resultant embryonic stem-like cells are induced to differentiate.
- 16. (Amended) The method of Claim 2, wherein the resultant embryonic stem-like cells are induced to differentiate.

A3

- 18. (Amended) Embryonic stem-like cells according to the method of Claim 1.
- 19. (Amended) Human embryonic stem-like cells according to the method of Claim 2.
- 21. (Amended) Human embryonic stem-like cells according to the method of Claim 4.

A4

- 22. (Amended) Human embryonic stem-like cells according to the method of Claim 6.
 - 23. (Amended) Human embryonic stem-like cells according to the method of claim 7.

AS

- 32. (Amended) The method of Claim 1, further comprising a step (v) whereby a gene is inserted, removed or modified in said embryonic stem-like cells.
- 33. (Amended) The method of Claim 32, wherein said gene encodes a therapeutic enzyme, a growth factor or a cytokine.

Atty Reference: 015837-0275817

Page 3

A

35. (Amended) The method of Claim 32, wherein said gene is removed, modified or deleted by homologous recombination.

Kindly add the following new claims prior to further examination:

- --51. The embryonic or stem-like cells of Claim 32.
- 52. The method of Claim 1, wherein said adult differentiated cell and said enucleated oocyte are phylogenetically dissimilar.
- 53. A method of producing an activated nuclear transfer unit capable of being cultured to a size of at least two cells, wherein said nuclear transfer unit comprises mitochondria from a species other than said adult differentiated cell, comprising:
- (i) inserting a human or mammalian cell or cell nucleus from an adult differentiated cell of a first species into an enucleated oocyte of a second species under conditions suitable for formation of a nuclear transfer (NT) unit; and
- (ii) activating the resultant NT unit so as to produce an activated nuclear transfer unit capable of being cultured to a size of at least two cells.
- 54. The method of Claim 53, wherein said activated nuclear transfer unit is capable of being cultured to 2 to 400 cells.
- 55. The method of Claim 53, wherein the adult cell inserted into the enucleated animal oocyte is a human cell, and the enucleated oocyte is obtained from an ungulate.
 - 56. The method of Claim 55, wherein said ungulate is a bovine.
- 57. An activated nuclear transfer unit obtained according to the method of Claim 53.--



Amendment and Response U.S. Serial No. 09/260,468

Attorney Reference: 015837-0275817

Page 2

isolating cells from said disassociated nuclear transfer unit to obtain (v) embryonic stem-like cells.

See the attached Appendix for the changes made to effect the above claim(s).

Please add the following new claim(s):

- (New) The method of claim 1, wherein said cells isolated from said disassociated nuclear transfer unit are isolated from cells originating from the inner-most portion of said nuclear transfer unit.
 - (New) An embryonic stem-like cell isolated from the inner-most portion of a nuclear 59. transfer unit according to the method of claim 58.
- (New) The method of claim 1, wherein the adult cell inserted into the enucleated 60. animal oocyte is a human cell, and the enucleated oocyte is a primate oocyte.
- (New) The method of claim 53, wherein the adult cell inserted into the enucleated 61. animal oocyte is a human cell, and the enucleated oocyte is a primate oocyte.--

WHAT IS CLAIMED IS:

A method of producing embryonic or stem-like cells comprising the following steps:

- inserting a desired differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell under conditions suitable for the formation of a nuclear transfer (NT) unit;
 - (ii) activating the resultant nuclear transfer unit;
- (iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and
- (iv) culturing cells obtained from said cultured NT units to obtain embryonic or stem-like cells.
- 2. The method of Claim 1, wherein the cell inserted into the enucleated animal oocyte is a human cell.
 - 3. The method of Claim 2, wherein said human cell is an adult cell.
- 4. The method of Claim 2, wherein said human cell is an epithelial cell, keratinocyte, lymphocyte or fibroblast.
- 5. The method of Claim 2, wherein the oocytes are obtained from a mammal.

5. A.

)

- 6. The method of Claim 5, wherein the animal oocyte is obtained from an ungulate.
- 7. The method of Claim 6, wherein said ungulate is selected from the group consisting of bovine, ovine, porcine, equine, capine, and buffalo.
- 8. The method of Claim 1, wherein the enucleated oocyte is matured prior to enucleation.
- 9. The method of Claim 1, wherein the fused nuclear transfer units are activated *in vitro*.
- 10. The method of Claim 1, wherein the activated nuclear transfer units are cultured on a feeder layer culture.
- 11. The method of Claim 10, wherein the feeder layer comprises fibroblasts.
- 12. The method of Claim 1, wherein in step (iv) cells from a NT unit having 16 cells or more are cultured on a feeder cell layer.
- 13. The method of Claim 12, wherein said feeder cell layer comprises fibroblasts.
- 14. The method of Claim 13, wherein said fibroblasts comprise mouse embryonic fibroblasts.

The method of Claim 1, wherein the resultant embryonic or stemlike cells are induced to differentiate.

- The method of Claim 2, wherein the resultant embryonic or stem-16. like cells are induced to differentiate.
- The method of Claim 1, wherein fusion is effected by electrofu-17. sion.

Embryonic or stem-like cells obtained according to the method of Claim 1.

- Human embryonic or stem-like cells obtained according to the 19. method of Claim 2.
- Human embryonic of stem-like cells obtained according to the 20. method of Claim 3.

Human embryonic or stem-like cells obtained according to the method of Claim 4.

- Human embryonic or stem-like cells obtained according to the 22. method of Claim 6.
- 23. Human embryonic or stem-like cells obtained according to the method of Claim 7.

- 24. Differentiated human cells obtained by the method of Claim 16.
- 25. The differentiated human cells of Claim 24, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells.
- 26. A method of therapy which comprises administering to a patient in need of cell transplantation therapy isogenic differentiated human cells according to Claim 24.
- 27. The method of Claim 26, wherein said cell transplantation therapy is effected to treat a disease or condition selected from the group consisting of Parkinson's disease, Huntington's disease, Alzheimer's disease, ALS, spinal cord defects or injuries, multiple sclerosis, muscular dystrophy, cystic fibrosis, liver disease, diabetes, heart disease, cartilage defects or injuries, burns, foot ulcers, vascular disease, urinary tract disease, AIDS and cancer.
- 28. The method of Claim 26, wherein the differentiated human cells are hematopoietic cells or neural cells.
- 29. The method of Claim 26, wherein the therapy is for treatment of Parkinson's disease and the differentiated cells are neural cells.
- 30. The method of Claim 26, wherein the therapy is for the treatment of cancer and the differentiated cells are hematopoietic cells.

31. The differentiated human cells of Claim 24, which contain and express an inserted gene.

- The method of Claim 1, wherein a desired gene is inserted, removed or modified in said embryonic or stem-like cells.
- 33. The method of Claim 32, wherein the desired gene encodes a therapeutic enzyme, a growth factor or a cytokine.
- 34. The method of Claim 32, wherein said embryonic or stem-like cells are human embryonic or stem-like cells.
- 35. The method of Claim 32, wherein the desired gene is removed, modified or deleted by homologous recombination.
- 36. The method of Claim 1, wherein the donor cell is genetically modified to impair the development of at least one of endoderm, ectoderm and mesoderm.
- 37. The method of Claim 1, wherein the donor cell is genetically modified to increase differentiation efficiency.
- 38. The method of Claim 36, wherein the cultured nuclear transfer unit is cultured in a media containing at least one capsase inhibitor.

p. 1

- 39. The method of Claim 1, wherein the donor cell expresses a detectable label that is indicative of the expression of a particular cyclin.
- 40. The method of Claim 36, wherein the donor cell has been modified to alter the expression of a gene selected from the group consisting of SRF, MESP-1, HNF-4, beta-1, integrin, MSD, GATA-6, GATA-4, RNA helicase A, and H beta 58.
- 41. The method of Claim 37, wherein said donor cell has been genetically modified to introduce a DNA that provides for expression of the Q7 and/or Q9 genes.
- 42. The method of Claim 41, wherein said gene or genes are operably linked to a regulatable promoter.
- 43. The method of Claim 1, wherein the donor cell has been genetically modified to inhibit apoptosis.
- 44. The method of Claim 43, wherein reduced apoptosis is provided by altering expression of one or more genes selected from the group consisting of Bad, Bok, BH3, Bik, Blk, Hrk, BNIP3, Gim_L, Bid, EGL-1, Bcl-XL, Bcl-w, Mcl-1, A1,Nr-13, BHRF-1, LMW5-HL, ORF16, Ks-Bcl-2, E1B-19K, and CED-9.
- 45. The method of Claim 44, wherein at least one of said genes is operably linked to an inducible promoter.

- 46. A mammalian somatic cell that expresses a DNA that encodes a detectable marker, the expression of which is linked to a particular cyclin.
- 47. The cell of Claim 46, wherein the cyclin is selected from the group consisting of cyclin D1, D2, D3, B1, B2, E, A and H.
- 48. The cell of Claim 46, wherein the detectable marker is a fluorescent polypeptide.
- 49. The cell of Claim 48, wherein said mammalian cell is selected from the group consisting of human, primate, rodent, ungulate, canine, and feline cells.
- 50. The cell of Claim 48, wherein said cell is a human, bovine or primate cell.

Add